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High HMGB1 level is associated with poor outcome of septicemic melioidosis

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SUMMARY

Objectives: A high level of HMGB1 (high-mobility group box 1) – a late onset inflammatory mediator – is a marker of lethal sepsis in several infectious diseases. The level of HMGB1 in the plasma of *Burkholderia pseudomallei*-infected patients was investigated together with the severity of the disease. The neutralization of HMGB1 to improve survival was also tested in a mouse model.

Methods: HMGB1 levels in the plasma of 77 septic patients, 40 with *B. pseudomallei* infection and 37 with other bacterial infections, were determined by ELISA. Neutralizing antibody against purified recombinant HMGB1 was prepared in rabbits (rab-a-HMGB1) and its potential as an adjunct therapy was evaluated in *B. pseudomallei*-infected Balb/c mice treated with suboptimal doses of ceftazidime.

Results: The plasma from *B. pseudomallei*-infected patients showed significantly higher HMGB1 levels than the plasma from other septic patients (median 11.1 ng/ml vs. 7.1 ng/ml). The HMGB1 level was significantly higher in patients with melioidosis who died than in those who survived (median 14.8 ng/ml vs. 9.2 ng/ml). Moreover, the HMGB1 level was significantly correlated with the clinical severity score (SOFA score). In the mouse study, although the rab-a-HMGB1 by itself could not improve the survival outcome of *B. pseudomallei*-infected mice, it could nevertheless enhance the effectiveness of suboptimal doses of ceftazidime in the treatment of these animals.

Conclusion: The level of HMGB1 in septic melioidosis patients can be used as a marker of late severe sepsis. Neutralizing antibody to HMGB1 may be used as an adjunct therapy to improve the outcome of melioidosis.

1. Introduction

HMGB1 (high-mobility group box 1), also known as high-mobility group protein 1 (HMG-1), is a 30-kD non-histone nuclear protein functioning as a transcriptional regulator essentially for cellular physiology.[1,2] Under certain pathological insults, such as inflammation or necrotic cell death, HMGB1 transmigrates from the nucleus to the outside of cells, where it functions as a potent proinflammatory mediator by interacting with toll-like receptors TLR-2, -4, or the receptor for advanced glycation end-products (RAGE) presenting on other cells.[2,3] In the scenario of sepsis in humans, HMGB1, as well as many other pro- and anti-inflammatory cytokines, is released into the circulation and participates in the pathogenesis, but with kinetics distinctly different from the others. The kinetics of HMGB1 release during sepsis is relatively slow and thus it is classified as a late onset mediator of sepsis. The few sepsis mediators known so far grouped into this classification include HMGB1, macrophage migration inhibitory factor (MIF), and complement factor 5a (C5a).[4,5] A feature of late onset mediators in releasing kinetics makes HMGB1 a promising factor to be targeted as an adjunctive therapy in the treatment of sepsis.[6,7] In animal sepsis models using cecal ligation and puncture

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(CLP), the administration of HMGB1-specific neutralizing antibodies has been shown to increase animal survival rates.[8,9]

In northeastern Thailand, one of the most common etiologic organisms for community-acquired septicemia is *Burkholderia pseudomallei*, a Gram-negative bacterium that naturally inhabits soil.[10] Although infection by this organism can manifest in various clinical forms, the most serious one, which is not uncommon, is the septicemic form. The mortality rate of patients with the septicemic form of *B. pseudomallei* infection, or so-called septicemic melioidosis, has been shown to be as high as 40%, despite the use of appropriate antibacterial agents.[11] It has been shown previously that many pro- and anti-inflammatory cytokines, such as interleukin (IL)-6, IL-10, etc. play a role in the pathological processes of septicemic melioidosis, as well as having a predictive value with regard to the clinical outcome.[12,13]

In order to determine whether or not there is a relationship between HMGB1 and septicemic melioidosis, we performed a prospective observational study on patients in the early stages of clinical sepsis who were likely to have *B. pseudomallei* infection as the etiology. These patients were hospitalized in two provincial general hospitals (300 beds) in northeastern Thailand. As a relationship between HMGB1 and survival was confirmed in these patients, it was hypothesized that neutralization of serum HMGB1 might have a value in treating septicemic melioidosis. Therefore an experimental mouse model was employed to determine whether the neutralization of HMGB1 with polyclonal antibodies alone or when used in conjunction with suboptimal doses of ceftazidime could improve survival in cases of infection.

2. Materials and methods

2.1. Patients

Adult patients with community-acquired sepsis likely to have an etiology of *B. pseudomallei*, who were newly admitted to the hospitals of Nong Khai and Mahasarakham during 2008–2010, were included in this study. These two hospitals are located in the upper region of northeastern Thailand, an area with a high prevalence of human melioidosis.[14]

Clinical sepsis was defined as the appearance of systemic inflammatory response syndrome (SIRS) with a report of at least one positive culture from a body fluid specimen.[15] SIRS was confirmed in the presence of two or more of the following: a body temperature >38 °C or <36 °C, a heart rate >90 beats per min, a respiratory rate >20 breaths per min or the necessity for respiratory assistance, and a white blood cell count $>12 \times 10^9$ cells/l or the presence of >10% band form neutrophils. Since it had been reported in a previous local survey that *B. pseudomallei* infection accounted for approximately 5–10% of community-acquired sepsis, only those cases with a clinical appearance highly suggestive of melioidosis were enrolled. Patients with a high probability of melioidosis included those with a history of environmental exposure, rapid clinical progression, the presence of diabetes, or otherwise a clinical manifestation that was not indicative of other certain organisms. Despite this selection process, when the culture results were reported, approximately half of the enrolled patients were not infected with *B. pseudomallei*; their plasma samples were studied for HMGB1 and they were placed in a non-*B. pseudomallei* sepsis group. Patients who were culture-negative or had a known history of being immunocompromised (e.g. HIV or cancer chemotherapy), were excluded from the study. The types of positive specimen, bacteria isolated, and the percentage of each infection are shown in Table 1.

Soon after enrollment and with signed informed consent, venous blood was collected in ethylenediaminetetraacetic acid (EDTA) and the plasma was then separated and stored at −80 °C until the HMGB1 measurement was performed. The clinical severity at the time of blood sample collection was assessed with the SOFA score (Sequential Organ Failure Assessment).[16] The study protocol was approved by the ethics committees of the Ministry of Public Health (Ref. 103/2551) and Khon Kaen University (Ref. HE510905).

Table 1
Types of specimens and bacterial identification in all patients

Bacteria identified	*n* (%)	Specimens (*n*)
Burkholderia pseudomallei	40 (51.9%)	Blood (28), blood + sputum (3), sputum (7), pus (1), pleural fluid (1)
Klebsiella pneumoniae	11 (14.3%)	Sputum (8), blood (3)
Escherichia coli	10 (13.0%)	Blood (8), sputum (2)
Pseudomonas aeruginosa	4 (5.2%)	Sputum (1), blood (1), pus + sputum (1), urine (1)
Acinetobacter spp	3 (3.9%)	Blood (2), sputum (1)
Enterococcus	3 (3.9%)	Sputum (2), pus (1)
Beta-hemolytic streptococci	2 (2.6%)	Blood (2)
Staphylococcus aureus	2 (2.6%)	Blood (2)
Salmonella spp	1 (1.3%)	Blood (1)
Streptococcus pneumoniae	1 (1.3%)	Sputum (1)

2.2. Rabbit anti-mouse HMGB1 polyclonal antibodies

A commercially available mouse HMGB1 expression vector (pReceiver-B11x; Capital Bioscience, Rockville, MD, USA) was transfected into *Escherichia coli* DH5-α. Recombinant protein was then expressed and purified using Ni-Sepharose (HisTrap FF; GE Healthcare, Pittsburg, PA, USA). Polyclonal antibodies were produced in a female New Zealand rabbit immunized four times (days 0, 14, 28, and 42) with subcutaneous injections of 0.5 mg recombinant protein along with complete Freund's adjuvant (CFA) for the first injection and incomplete Freund's adjuvant (IFA) for the others, according to the protocol described by Lin et al.[17] The antibodies were then obtained by purification using ammonium sulfate precipitation, followed by Hi-Trap Protein G Affinity column and Hi-Trap Desalting columns (GE Healthcare Pittsburg, PA, USA), as per the manufacturer's instructions. The reactivity of the antibodies produced against mouse HMGB1 was confirmed by probing with the recombinant protein in a Western blot and comparison with other commercially available rabbit polyclonal antibodies against HMGB1 (Abcam, Cambridge, MA, USA). The latter confirmed the sequence homology of the constructed recombinant protein (Figure 1).

2.3. Mouse model of B. pseudomallei infection

Six- to eight-week-old BALB/c mice were obtained from the National Laboratory Animal Center, Mahidol University. Experiments were done with the ethical approval of the Animal Ethics Committee of the Faculty of Medicine, Khon Kaen University (Ref. AE04/53). At the time-point −2 h, mice were treated with either polyclonal anti-mouse HMGB1 (rab-a-HMGB1) or isotype control antibodies at 2 mg/mouse via an intraperitoneal injection. At time-point 0 h, mice were then challenged intraperitoneally with a highly virulent *B. pseudomallei* strain A2 at inoculum sizes of 3–10 LD_{50} (median lethal dose). At indicated time-points, aliquots of mouse blood were taken from the periorbital plexus.

2.4. Protocol for adjunct treatments

Animals were infected intraperitoneally with a 5-LD_{50} log phase culture of *B. pseudomallei* in a volume of 100 μl (day 0). They were then divided randomly into three groups (four per group). The animals received either phosphate buffered saline (PBS) (control), 2 mg of rab-a-HMGB1 plus ceftazidime (1/4 of the optimal dose for

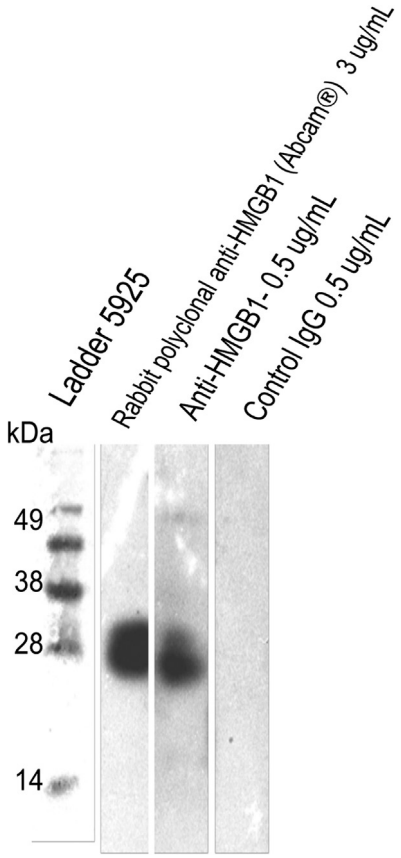

Figure 1. Western blot analysis of rabbit polyclonal antibodies against HMGB1: the recombinant mouse HMGB1 (1 μg) as separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane by Western blot. Reactivity of produced antibodies against mouse HMGB1 was probed with goat anti-rabbit horseradish peroxidase (HRP) (Invitrogen, Camarillo, CA, USA); chemiluminescent HRP substrate (Thermo Scientific, Rockford, IL, USA) was then added. Commercially available rabbit polyclonal antibodies against HMGB1 (Abcam, Cambridge, MA, USA) and pre-immune rabbit IgG were used as negative controls.

mice, 300 mg/kg)[18] (a-HMGB1/Ctz), or 2 mg of isotype control (normal rabbit IgG) plus ceftazidime (300 mg/kg) (isotype/Ctz) 2 h before being infected. Ceftazidime (300 mg/kg) was administered intraperitoneally twice daily for 10 days (Table 2). Survival rates of the mice were observed for 14 days. All studies were done in two independent experiments.

2.5. Determination of HMGB1

The HMGB1 levels in plasma in both humans and mice were subsequently measured using commercially available ELISA kits (IBL International GmbH, Hamburg, Germany).

Table 2
Protocol for adjunct treatments[a]

Group No.	No. of mice	Adjunct treatment with:[b]	Antibiotic treatment with:[c]
I: Control	4	PBS	None
II: a-HMGB1/Ctz	4	Rabbit IgG anti-HMGB1	Ceftazidime
III: Isotype/Ctz	4	Normal rabbit IgG	Ceftazidime

PBS, phosphate buffered saline.
[a] All mice were infected intraperitoneally with 5 LD_{50} of *Burkholderia pseudomallei* on day 0.
[b] 2 mg/mouse of antibodies or IgG was given intraperitoneally 2 h before infection.
[c] 300 mg/kg of ceftazidime was given on day 1 and twice daily for 10 days.

2.6. Statistical analysis

The statistical significance of differences was evaluated using the Student's *t*-test, Pearson Chi-square test, or Mann–Whitney *U*-test. Statistical significance was defined as $p < 0.05$.

3. Results

Seventy-seven adult patients with clinically documented sepsis (SIRS), plus a retrospective positive culture from any body fluid specimen, were enrolled into the study; 40 had *B. pseudomallei* sepsis (*B. pseudomallei* sepsis group) and the other 37 had other bacterial sepsis (non-*B. pseudomallei* sepsis group). Clinical parameters in the two groups are shown in Table 3.

Patients in the *B. pseudomallei* sepsis group were found to be significantly younger than those in the non-*B. pseudomallei* sepsis group (mean 49.8 years vs. 60.3 years; $p < 0.001$); this finding is in accordance with previous reports,[19] supporting that the acquisition of *B. pseudomallei* requires environmental exposure, which is usually more frequent in persons of actively working age. Severity parameters, in terms of 1-month death rates and SOFA scores, were not different between the two groups. *B. pseudomallei* sepsis patients had a higher number of reports showing a positive blood culture. Among those with non-*B. pseudomallei* sepsis, the causative organisms and their frequencies are shown in Table 1.

For the determination of HMGB1 levels, patient venous blood samples were collected as soon as possible once clinical sepsis was confirmed, regardless of culture results at the time, with a mean ± standard deviation (SD) interval after clinical onset of 2.8 ± 1.8 days for the *B. pseudomallei* sepsis group and 1.5 ± 0.9 days for the non-*B. pseudomallei* sepsis group. HMGB1 measurements showed that patients with *B. pseudomallei* sepsis had median plasma HMGB1 levels significantly higher than non-*B. pseudomallei* sepsis patients (11.1 ng/ml vs. 7.1 ng/ml, $p = 0.001$, Mann–Whitney *U*-test; Figure 2A). Among those with *B. pseudomallei* sepsis, plasma HMGB1 levels were higher in those with a 1-month outcome of death than in those who survived (median 14.8 ng/ml vs. 9.2 ng/ml, $p = 0.038$; Figure 2B). The relationship between 1-month death outcome and HMGB1 was also observed when considering the two groups as a whole (data not shown). A significant but weak correlation between SOFA scores and HMGB1 levels among subjects overall was observed in this study (Spearman's rho = 0.236, $p = 0.041$; Figure 2C).

As a relationship between HMGB1 and survival was confirmed in these patients, it was hypothesized that neutralization of mouse HMGB1 may improve survival in the mouse model of septicemic melioidosis. To test this hypothesis, a batch of rabbit polyclonal antibodies against mouse HMGB1 was produced. Anti-HMGB1 antibodies or IgG for controls at 2 mg per mouse was injected via intraperitoneal route 2 h prior to challenge with the highly virulent *B. pseudomallei* strain A2, also via the intraperitoneal cavity at an

Table 3
Clinical observations (SOFA score) and details of patients included in this study ($N = 77$)

	Overall	*Bp* sepsis	Non-*Bp* sepsis	*p*-Value
Number of patients	77	40	37	
Age, years, mean ± SD		49.8 ± 12.8	60.3 ± 12.9	<0.001[b]
Male, *n* (%)		31 (77.5%)	28 (75.7%)	0.85[c]
SOFA score, mean ± SD		6.3 ± 4.8	6.0 ± 3.6	0.133[b]
Death[a], *n* (%)		16 (40.0%)	12 (32.4%)	0.490[c]
Blood culture positive, *n* (%)		32 (80.0%)	19 (51.4%)	0.008[c]

SOFA, Sequential Organ Failure Assessment; *Bp*, *Burkholderia pseudomallei*; SD, standard deviation.
[a] Death within 1 month.
[b] Student's *t*-test.
[c] Pearson Chi-square test.

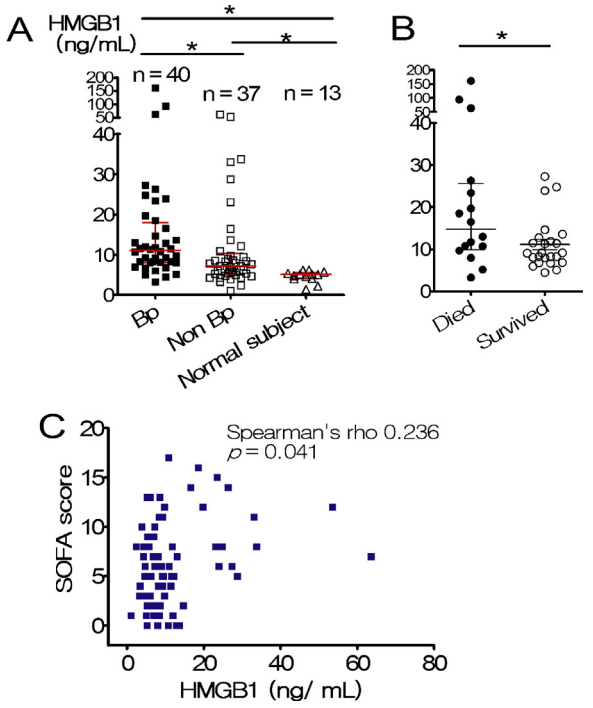

Figure 2. HMGB1 levels in the *Burkholderia pseudomallei* group and the other bacterial infections group. The plasma of patients with melioidosis and patients with other bacterial infections was collected as soon as possible after sepsis appeared clinically, regardless of the culture results at the time, and the HMGB1 level was detected by ELISA (A). The levels of HMGB1 were compared between patients with melioidosis who died and those who survived (B). The correlation between the SOFA score and the *B. pseudomallei* and non-*B. pseudomallei* sepsis groups is shown (C). Asterisks (*) indicate a significant difference ($p < 0.05$).

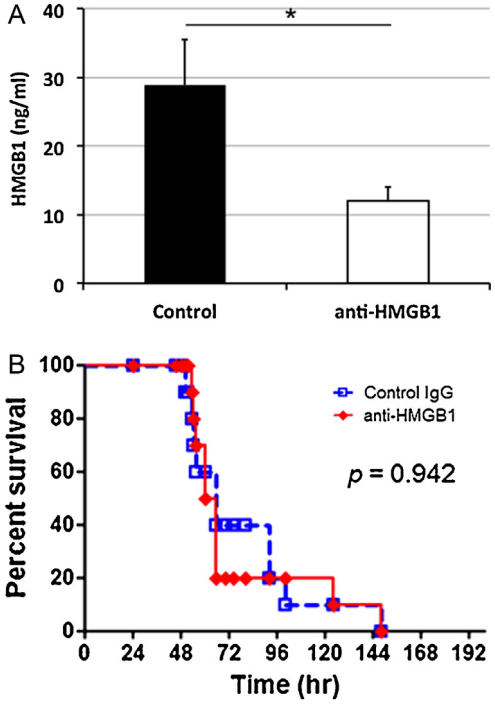

Figure 3. The survival rate and HMGB1 levels in mice with melioidosis. Anti-HMGB1 antibodies or control IgG at 2 mg per mouse was injected via intraperitoneal route 2 h prior to challenge with 3 LD_{50} of *Burkholderia pseudomallei* strain A2 via intraperitoneal route. The plasma HMGB1 levels in both groups were detected at 52 h post infection by ELISA (A). The survival patterns of the two treatment arms are shown (B). The asterisk (*) indicates a significant difference ($p < 0.05$).

inoculum size of 3 LD_{50}. Despite the fact that HMGB1 could be neutralized, as shown by the plasma HMGB1 levels, which were significantly decreased at 52 h post infection in the anti-HGMB1 treatment group (Figure 3A, $p = 0.035$), the survival patterns of the two treatment groups were not different (Figure 3B, $p = 0.942$). In both treatment arms, *B. pseudomallei* killed mice as early as 48 h, and all mice died within 6 days. Similar survival rates were observed in two other experiments, one using an inoculum size of 10 LD_{50} and the other using a different time schedule for anti-HMGB1 injection (two injections of 0.5 mg/mouse, at post infection times of 20 h and 39 h; data not shown). Therefore, it can be summarized that neutralization of HMGB1 failed to improve survival in a severe sepsis model of *B. pseudomallei* infection.

When anti-HMGB1 was used as an adjuvant therapy, the a-HMGB1/Ctz group had a 30% survival rate, whereas all mice in the untreated control group and in the isotype/Ctz group were dead within 11 days (Figure 4). These results indicate that the anti-HMGB1 had the capability of improving the outcome of the *B. pseudomallei*-infected mice receiving antibiotic therapy.

4. Discussion

There is growing evidence that late onset inflammatory mediators may play an important role in the pathological process of sepsis. One of the most common manifestations of *B. pseudomallei* infection is highly fatal clinical sepsis. It was therefore hypothesized that HMGB1, which is a late onset inflammatory mediator, might have a role in septicemic melioidosis. Recently, MIF, another late onset mediator of sepsis, was shown to participate in the pathogenesis of human melioidosis as its expression levels correlated with the clinical outcome.[20]

In the current study, it was demonstrated that, in a similar pattern to that shown elsewhere, plasma HMGB1 was increased in sepsis in both *B. pseudomallei* infection and in other non-specific bacterial infections. A remarkable distinction between these different etiologies, however, was the higher levels of HMGB1 in *B. pseudomallei* infections. If one classifies sepsis of organisms other

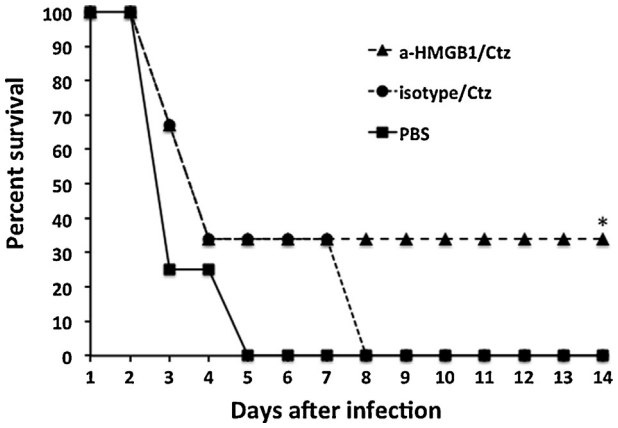

Figure 4. Effect of suboptimal antibiotic doses with/without anti-HMGB1 on survival rates in the treatment of *Burkholderia pseudomallei*-infected mice. Three groups of mice (four mice/group; groups I–III) were infected with 5 LD_{50} of *B. pseudomallei* A2 on day 0. The treatments were then performed on groups II and III. Groups II and III were treated with 300 mg/kg of ceftazidime with 2 mg of rabbit IgG anti-HMGB1 or 2 mg of normal rabbit IgG 2 h, respectively, before infection. Ceftazidime was administered intraperitoneally twice daily for 10 days. Group I was treated with phosphate buffered saline (PBS) as control. Mice were observed daily and the percentage of surviving mice in all groups plotted against time. Data represent the results of two independent experiments. The asterisk (*) indicates a significant difference ($p < 0.05$).

than *B. pseudomallei* as a common pool of pathological etiology, it is possible that the higher HGMB1 levels in *B. pseudomallei* sepsis contributed to some degree to the pathological differences. One possible pathological difference is the difference in kinetics of bacterial multiplication, together with differences in kinetics of host responses opposing the bacterial multiplication and the tissue destruction. Differences in the percentage of subjects with positive blood cultures (Table 1), or in other words differences in the numbers of organisms making up the bacteremia in an equal blood volume between the *B. pseudomallei* sepsis and the non-*B. pseudomallei* sepsis groups, is another line of evidence supporting the role of host–pathogen interaction kinetics mentioned above. It has also been shown previously, in a similar scenario of clinical sepsis, that the number of bacteria per milliliter blood volume in *B. pseudomallei* infection differs from the number of other bacteria.[21]

With regard to HMGB1 levels and their clinical associations, HMGB1 levels in *B. pseudomallei* sepsis were found to be associated with 1-month mortality outcomes. This finding is in accordance with those of previous reports describing other organisms in sepsis,[2] as well as in the non-*B. pseudomallei* group in the present study. Thus, it can be proposed that HMGB1 is another mediator that has an association with clinical outcomes, particularly in mortality outcomes. Nevertheless, the association with clinical severity in terms of the correlation of SOFA scores and HMGB1 levels was found to be less prominent when compared with the association of HMGB1 with the mortality outcome.

With the findings in humans as demonstrated, it could be hypothesized that targeting HMGB1 in an animal model of septicemic melioidosis would improve animal survival. In this respect, a mouse model of acute sepsis with *B. pseudomallei* was used in which BABL/c mice were infected via an intraperitoneal injection of a highly virulent *B. pseudomallei* strain. Under these infection conditions with this bacterial strain, wherein the LD_{50} within 1 month was only at 20 CFU,[22] the mouse would develop lethality within 3 days when the inoculum sizes were of $10 \times$ to $20 \times LD_{50}$, and thus this infection scenario would represent acute sepsis rather than chronic infection. In order to prevent rapid stimulation of HMGB1 after infection, the anti-HMGB1 was injected 2 h before bacteria were given. This design is similar to that used in a previous study using anti-MIF in the treatment of the mouse with melioidosis.[19] Nevertheless, when this model was applied, HMGB1 neutralization with rabbit polyclonal antibodies failed to demonstrate protective effects of the treatment, although such antibodies could significantly reduce HMGB1 levels (Figure 3A). The failure of protection might be due to the overwhelming bacteremia in treated animals (data not shown).

Therefore the use of ceftazidime, one of the drugs of choice for melioidosis, together with anti-HMGB1 as an adjunct therapy was tested. The limited results demonstrated that the use of rab-a-HMGB1 as an adjunct therapy led to a 30% survival rate, whereas all mice in the control and untreated groups were dead within 11 days. This anti-HMGB1 might lower the excessive and unregulated levels of proinflammatory cytokines while ceftazidime controls the bacteremia that leads to death. It is possible that with higher doses and/or higher neutralizing capacity of antibody, or more optimal administration procedures, a higher rate of success would be achieved. Ongoing experiments along these lines are being planned.

Before using the antibody to neutralize HMGB1, the protocol also employed a range of ethyl pyruvate treatments as a preliminary method for HMGB1 neutralization in the same animal model, but did not see any protective effects (data not shown). Ethyl pyruvate is a small organic molecule of metabolic intermediates shown previously in other models to have the ability to reduce HMGB1 release upon stimulation.[23,24] Treatment with ethyl pyruvate was also shown to improve survival in a sepsis model using CLP.[23,25] The reason for the discrepancy in the protective effect of HMGB1 neutralization between the present animal model of acute lethal *B. pseudomallei* infection and other models of sepsis, in a majority the CLP models, is currently unknown. Since the insulting organisms of CLP, which are likely to be commensal organisms, are clearly different from highly pathogenic organisms such as *B. pseudomallei*, particularly with respect to host immunity, one might speculate that the differences in host–pathogen interaction at the cellular level, especially in bacterial growth kinetics, is a possible reason for the discrepancy. Differences in bacterial growth kinetics in interactions with the host have been exemplified in an in vitro assay that showed that the virulent *B. pseudomallei* can continue to grow inside phagocytes, even at a relatively low multiplication of infection (MOI), i.e. at MOI of 1.[26] In contrast, in another similar assay system, this type of growth kinetics was found to be uncommon for *E. coli*, a representative of the commensal bacteria.[27] Differences at the cellular level of the host–pathogen interaction then lead to diversification of subsequent immunology and pathology, and thus may result in the discrepancy in the protective effect of HMGB1 neutralization.

In conclusion, the level of HMGB1 in septic melioidosis patients could be used as a marker of late severe sepsis. Although neutralizing antibody to HMGB1 itself could not protect mice from infection, it might be used as an adjunct therapy to improve the outcome of melioidosis.

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Conflict of interest: None.

References

1. Yang H, Tracey KJ. High mobility group box 1 (HMGB1). *Crit Care Med* 2005;**33**:S472–4.
2. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;**285**:248–51.
3. Yang H, Wang H, Czura CJ, Tracey KJ. The cytokine activity of HMGB1. *J Leukoc Biol* 2005;**78**:1–8.
4. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat Med* 2003;**9**:517–24.
5. de Jong HK, van der Poll T, Wiersinga WJ. The systemic pro-inflammatory response in sepsis. *J Innate Immun* 2010;**2**:422–30.
6. Wang H, Zhu S, Zhou R, Li W, Sama AE. Therapeutic potential of HMGB1-targeting agents in sepsis. *Expert Rev Mol Med* 2008;**10**:e32.
7. Mackenzie I, Lever A. Management of sepsis. *BMJ* 2007;**335**:929–32.
8. Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, et al. Reversing established sepsis with antagonist of endogenous high-mobility group box 1. *Proc Natl Acad Sci U S A* 2004;**101**:296–301.
9. Suda K, Kitagawa Y, Ozawa S, Saikawa Y, Ueda M, Ebina M, et al. Anti-high-mobility group box chromosome protein 1 antibodies improve survival of rats with sepsis. *World J Surg* 2006;**30**:1755–62.
10. Wiersinga WJ, van der Poll T, White NJ, Day NP, Peacock SJ. Melioidosis: insights into pathogenicity of *Burkholderia pseudomallei*. *Nat Rev Microbiol* 2006;**4**:272–82.
11. Leelarasamee A. Recent development in melioidosis. *Curr Opin Infect Dis* 2004;**17**:131–6.
12. Simpson AJ, Smith MD, Weverling GJ, Suputtamongkol Y, Angus BJ, Chaowagul W, et al. Prognostic value of cytokine concentrations (tumor necrosis factor-alpha, interleukin-6, and interleukin-10) and clinical parameters in severe melioidosis. *J Infect Dis* 2000;**181**:621–5.
13. Lauw FN, Simpson AJ, Prins JM, Smith MD, Kurimoto M, van Deventer SJ, et al. Elevated plasma concentrations of interferon (IFN)-gamma and IFN-gamma

inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. *J Infect Dis* 1999;**180**:1878–85.
14. Limmathurotsakul D, Wongrattanacheewin S, Teerawattanasook N, Wongsuvan G, Chaisuksant S, Chetchotisakd P, et al. Increasing incidence of human melioidosis in northeast Thailand. *Am J Trop Med Hyg* 2010;**82**:1113–7.
15. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al., Surviving Sepsis Campaign. International guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008;**36**:296–327.
16. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 1988;**26**:1793–800.
17. Lin Q, Fang J, Fang D, Li B, Zhou H, Su SB. Production of recombinant human HMGB1 and anti-HMGB1 rabbit serum. *Int Immunopharmacol* 2011;**11**:646–51.
18. Ulett GC, Hirst R, Bowden B, Powell K, Norton R. A comparison of antibiotic regimens in the treatment of acute melioidosis in a mouse model. *J Antimicrob Chemother* 2003;**51**:77–81.
19. Boonma P, Bhuripanyo K, Sithikaesorn J, Patjanasoontorn B, Sookparnee M. Discriminant analysis among septicemic melioidosis and other bacterial septicemia. *J Med Assoc Thai* 1990;**73**:543–7.
20. Wiersinga WJ, Calandra T, Kager LM, van der Windt GJ, Roger T, le Roy D, et al. Expression and function of macrophage migration inhibitory factor (MIF) in melioidosis. *PLoS Negl Trop Dis* 2010;**4**:e605.
21. Walsh AL, Smith MD, Wuthiekanun V, Suputtamongkol Y, Chaowagul W, Dance DA, et al. Prognostic significance of quantitative bacteremia in septicemic melioidosis. *Clin Infect Dis* 1995;**21**:1498–500.
22. Panomket P, Chetchotisakd P, Sermswan RW, Pannengpetch P, Wongratanacheewin S. Use of a low dose steroid as adjunctive treatment in mouse severe sepsis due to *Burkholderia pseudomallei* infection. *Ann Trop Med Parasitol* 2009;**103**:635–46.
23. Fink MP. Ethyl pyruvate: a novel treatment for sepsis and shock. *Minerva Anestesiol* 2004;**70**:365–71.
24. Davé SH, Tilstra JS, Matsuoka K, Li F, DeMarco RA, Beer-Stolz D, et al. Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. *J Leukoc Biol* 2009;**86**:633–43.
25. Ulloa L, Ochani M, Yang H, Tanovic M, Halperin D, Yang R, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. *Proc Natl Acad Sci U S A* 2002;**99**:12351–6.
26. Charoensap J, Utisincharoens P, Engering A, Sirisinha S. Differential intracellular fate of *Burkholderia pseudomallei* 844 and *Burkholderia thailandensis* UE5 in human monocyte-derived dendritic cells and macrophages. *BMC Immunol* 2009;**10**:20.
27. Sukumaran SK, Shimada H, Prasadarao NV. Entry and intracellular replication of *Escherichia coli* K1 in macrophages require expression of outer membrane protein A. *Infect Immun* 2003;**71**:5951–61.